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NUTRITION REVIEWS

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NUTRITION REVIEWS

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RESOURCES IN NUTRITION RESEARCH*

Human nutrition is perhaps the largest and most complex scientific and social problem of our times. We are often asked what is the relation between public health nutrition and the nutrition programs of agricultural agencies. I know of no better answer to this question than the Statement of the Joint Committee on Nutrition of the Food and Agriculture Organization and the World Health Organization (*FAO, 1949*):

"There are differences in emphasis in the approach to nutrition in FAO and WHO. The objective of food for health is common to both, but in FAO the emphasis is on nutrition in relation to the production, distribution, and consumption of food, while in WHO the emphasis will be on nutrition in relation to the maintenance of health and the prevention of disease. Nevertheless, nutrition is a single whole which cannot be broken into a series of separate and discrete categories. Almost every practical program of nutrition has aspects which fall within the fields of interest of both FAO and WHO. Collaboration must therefore be flexible and no sharp dividing lines of responsibility can be drawn."

Nutritional science is the basis of any and all practical improvements in human nutrition. Hence, the combined efforts of private and public agencies in nutrition research are essential for the world's health and economy.

If the United States is to fulfill its international responsibilities, we must have increased support from all sources for scientific research leading to the alleviation of hunger and sickness. One of our great assets in the current world struggle is our relatively advanced position in the agricultural and nutritional sciences. To maintain that leadership, to attain a higher level of health for our own people, and to assist in increasing the world's productivity, we should set our sights high and strive for further advances through increased nutritional research.

The science of nutrition is relatively young. Yet its achievements—particularly in the past decade—have been so impressive that experts in all related fields predict much higher levels of health than we now have through the application of nutritional science.

As in other fields of research, the rate of advance in nutritional science depends in major part upon the emphasis we are willing to place upon basic studies—as contrasted with applied and developmental research—and upon the extent of support from all sources devoted to such investigations.

We look to our universities, medical schools, hospitals, and qualified research institutes for the bulk of fundamental research in the health sciences. Prior to World War II, these institutions obtained the major support of their research programs from private foundations, individual philanthropists, and industry. During the war, wide support of medical research became a matter of national policy and the federal contribution through contracts increased impressively during that period.

Since the war, we have witnessed an amazing increase in support of medical and related research by both private and public sources. In the past ten years also, two new resources have come to the fore as prominent investors in health research, the national voluntary health agencies and industrial foundations. In 1947, the President's Scientific Research Board stressed the importance of the industrial foundation as the prototype

* Abstract of a talk given at the Seventh Annual Meeting of The National Vitamin Foundation, Inc., April 3, 1952.

of a new source of support for medical research.

The history of health progress shows conclusively that the fundamental approach to scientific research is a necessary one. Basic investigations, often undertaken with little promise of practical results, are actually the wellsprings of science and technologic advance. Unless these springs are constantly fed, the likelihood of new practical advances is decreased. Many of the tremendous accomplishments which this country made during World War II were based upon fundamental research conducted over the years largely in other countries. Having learned the lesson that we must become as self-sufficient in basic science as we have in other areas, we have given increasing attention to fundamental research.

The complexity of science itself has made research increasingly expensive. Some of the new technics which have been devised for further advances against the unknown require facilities, equipment, supplies, and skills entirely beyond the reach of the lone, individual investigator or any one institution. For most of our universities and other nonprofit research institutions, these increased costs undeniably present difficulties. The significance of their problem has been generally recognized by leaders in industry and government, as well as by the nation as a whole. In all groups, there is awareness that research must be supported in increasing measure and that this support must come from both private and public sources. Action in response to that awareness is evident not only in the substantial growth of the private agencies' contribution, but also in their endorsement of governmental research programs and governmental aid to research.

There are a great number and a great variety of nutrition researches that could be singled out for comment, but through all would run the suggestion that increased knowledge of nutrition is fundamental to the solution of many of our major health problems. I am thinking particularly of those chronic disorders such as cancer and heart disease—disorders usually not associated in the public mind with nutrition. Moreover, it is known that nutritional disorders in early life may result years later in serious health problems. The corollary is that a proper diet contributes to healthful, productive adulthood and old age. Viewed in purely economic terms, the knowledge we have gained has made us a more productive people and has led to higher purchasing power and increased consumption. In short, nutrition research has played—and will continue to play—a tremendously vital role in raising the standard of life in America.

There are, of course, many unsolved problems still confronting us in nutrition—problems which will challenge our best efforts for years to come. We have not yet succeeded in identifying all the dietary factors which influence metabolism. There is much we do not understand about obesity, or about the influence of nutrition upon such chronic diseases as diabetes and arteriosclerosis. Certainly there is also need for more knowledge concerning food supplies themselves; how to utilize them to better advantage and how to extend them in the face of heavier demands which some sociologists believe might outstrip the world's capacity to produce.

I take a more optimistic view, one which I believe is shared by the majority of biologic scientists as well as by American industry. I believe that scientific effort and the application of its results offer modern man a chance to solve the problems of his physical and social environments. In the field of public health there is no doubt that our major contemporary problems are more complex and affect larger numbers of people than did the major problems of fifty years ago. It is equally true that science and its application have played the dominant roles in bringing about those changes. But even with our vastly augmented powers to transform new knowledge into products and services, there is no doubt that we are far

from applying all we know to the improvement of human health and welfare.

Today, we can be sure that science—including nutritional science—is laying the groundwork for another forward thrust in human health. If we are to benefit fully from the past achievements and the future promise of research, all elements of society—industry, government, the scientific community, and the public at large—must plan together and work together toward that goal.

Nutritional research has already accomplished one of the scientific miracles of our times in demonstrating what is required to nourish man. Now on the threshold of new, challenging discoveries, the young science of nutrition offers promise of a new era of better health comparable to that accompanying the earlier control of communicable diseases. It offers opportunities for immediate elevation of living standards and for continuing improvement in the health and strength of nations throughout the world. Those potentialities will be realized to the extent that scientists and institutions are supported in their endeavors to extend the frontiers of knowledge.

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MALNUTRITION IN YOUNG CHILDREN

The demonstration that a brief and transient period of specific vitamin deficiency in very young animals would induce widespread vascular lesions and hypertension in maturity, even though a normal diet was subsequently restored (W. S. Hartroft and C. H. Best, *Brit. Med. J.* **1,** *423 (1949)*; *Nutrition Reviews* **7,** *312 (1949)*) has added vigor to the interests of experimental and clinical nutritionists concerning the importance of adequate nutrition in young individuals. It is generally assumed that the nutritional state of infants has been greatly improved as a result of nutritional information supplied by hospitals and pediatricians. The nourishment of the school child (5 years old or more) similarly has been benefited by broad nutrition education programs in the schools. A recent report by J. P. Price and W. M. Hart (*J. Am. Med. Assn.* **148,** *5 (1952)*), however, indicated that a significant degree of malnutrition may exist in young children between the ages of 1 and 4 years.

Over a period of one year these workers studied 50 cases of malnutrition and anemia in children in this age group. The great majority were children whose parents were farmers and laborers in industrial plants, and were taken to the private offices of pediatrician or general practitioner. The usual chief complaints were that the child would not eat, tired easily, was restless at night, was a poor sleeper, or was frequently nervous or cross. On physical examination the most common finding was pallor with poorly developed muscles. Of the 50 children, 21 were underweight and 12 had clinical evidence of rickets. All 50 had hemoglobin levels below 10.5 g. per cent, with usually a significant reduction in the color index.

Careful nutritional histories were taken in each case. Forty-one of the 50 children, in spite of their relatively advanced age, were still receiving their milk from a nursing bottle. Several of these patients were more than 3 years old. The average consumption of milk per child, in twenty-four hours, ranged from 16 to 48 ounces. Associated with this relatively high milk intake was a corresponding reduction in the amount and variety of solid foods. Although cereal and fruit, with some meats and eggs, were on the dietary list of most of the children, amounts of these substances were significantly reduced and in many instances certain of them

had been stopped completely because the child found them unappetizing. An adequately well rounded intake of fresh vegetables was not present in any of the subjects. The supply of supplementary vitamins was similarly scant. The mothers of 35 children had given some vitamin preparation with more or less regularity, but this frequently ceased after the child had passed its first birthday. Examples of a day's diet of 3 of the children reveal in startling manner the absence of iron-containing foods and vegetables naturally rich in required vitamins.

The treatment for these malnourished and anemic children was directed primarily along five distinct lines: (1) The child was immediately weaned from the bottle or breast. (2) Specific instructions were given the parents with regard to the nature of a well-balanced diet and the child was encouraged to feed himself as much as possible. (3) An iron-vitamin mixture was administered regularly each day. In addition to the usual supplements of various B-complex components, the mixture was particularly rich in vitamins A and D, riboflavin and ascorbic acid. Thiamine hydrochloride was present in excessive amounts, in an attempt to stimulate the appetite. (4) The feces were studied for ova and parasites. (5) Further follow-up studies were done on the blood after treatment. In 25 of the patients whose parents followed instructions, the authors conclude that satisfactory results were obtained. In 4 of the 25 cases improvement was slow; in the remainder the response was described as immediate with an improvement in the child's color and appetite and a gain in hemoglobin of approximately 0.5 g. per cent per week on the average.

As a result of the response to therapy the authors have concluded that possibly the excessive intake of milk was the most important single factor in the development of malnutrition in these children. Failure to wean the baby from milk at the proper time may result in refusal of other food and subsequent development of a relatively persistent anemia. The second most important feature was probably the failure to continue the administration of supplementary vitamins beyond the first few months of life.

As is so often the case in pediatric practice, the proper treatment of the child included the proper education of the parents. Although they usually were informed of the need for milk and vitamins in the infant stage, many of them were not aware of the need for adequate supplementary materials, bulk elements and varied intake, once the child was beyond the infant stage. The fact that less than half of the children were underweight probably prevented many of the parents from appreciating a state of malnutrition in the child. The majority of the children were brought to the doctors' offices primarily for specific complaints and were seen to be in a state of malnutrition at their first visit. Had an adequate system of periodic examinations been instituted in the child's infant state, undoubtedly most of the malnutrition and anemia could have been prevented.

The inadequacy of body build, height, and weight as criteria of malnutrition is emphasized by a recent publication of T. D. Spies, S. Dreizen, G. S. Parker, and D. J. Silberman (*J. Am. Med. Assn.* **148,** *1376 (1952)*). The authors cite in detail a case of a patient who when first seen did not appear particularly abnormal for his age. He seemed well developed, but on careful physical examination he was found to have bilateral cheilosis, with glossitis and conjunctivitis. His parents stated that he frequently developed cracks or fissures in the corners of his mouth. A dietary history revealed a very poor intake of vegetables, with few meals containing milk, eggs, or meat. In order to test the value of this boy's food program, 60 rats were placed on the same diet, scaled down to rat requirements. Twelve animals were given the diet alone, with other groups receiving the basic diet and certain supplements. The rats receiving

only the child's food and water were extremely small, with poor hair growth and with soft, poorly developed muscles. Of all the animals studied, only the group receiving the child's diet plus casein, minerals and vitamins had normal growth curves.

If body development, height and weight of a young child seem to the parents to be within their own conception of normal standards, the child is frequently permitted to refuse an unwanted food, and to take a preferred substitute. When such a child is in the age group beyond the infant stage, the need for visits to the pediatrician is perhaps not apparent. Not until the age of 5 will the periodic check-up in school review the child's health status. The data discussed herein indicate that a serious degree of malnutrition may develop in these "undisciplined years" of ages 1 to 5, in spite of seeming well-being. These papers point up the present need for directed knowledge concerning specific details of an adequate diet for young children, and for periodic medical evaluation by qualified physicians, to be aimed particularly at parents of children between the infant stage and the school-age group.

COLD-WAR DIETARY RESTRICTIONS IN BERLIN

Observations on the food and nutritional situation in Berlin during and after the blockade have been reported by W. T. C. Berry, P. J. Cowin, and H. E. Magee (*Mo. Bull. Ministry of Health and Pub. Health Lab. Service* **10,** *155 (1951)*). The experiences undergone in Berlin are of potential interest in reference both to administrative and scientific aspects of food supply in civilian defense.

The blockade was enforced for some ten months (June 1948 to May 1949). Official rations were regarded as the only important source of food. The reader is not informed of the food and nutritional situation preceding the imposition of the blockade and thus lacks the "control" data with reference to which the information on food rations during the blockade is to be interpreted. Nor is he acquainted with the development of the strange dietary pattern exhibited by the rations in the early phase of the blockade.

There were three caloric groupings for adults (heavy workers, light workers and white-collar workers), with ration intakes of 2500, 2000, and 1600 calories. The authors noted that the highest caloric group included many individuals who were not working full time and many individuals doing light or sedentary work (clerks, administrators, actors and trade union officials). In addition to the physiologically unsound job classification, the dietary picture becomes further complicated by the fact that many of the workers obtained an extra meal on five or six days a week. The children received a meal in school. Under these conditions the caloric values of official rations have little meaning and are apt to be grossly misleading.

Serious errors have crept into the rationing of food for children. The rations for very young children (up to one year) were large (1790 calories) and decreased in higher age groups, with food rations of 1650, 1620 and 1560 calories for the age groups 1 to 6, 7 to 9, and 9 to 14 years. An exactly opposite trend would have been justified physiologically, with additional rations for the males during adolescence. The nutritional needs of pregnancy and lactation were considered. Mothers were placed in the second caloric group (2000 calories) from the fifth month to the end of pregnancy and received daily 500 ml. of milk during this period as well as for four months after the birth of a child.

Qualitatively, the main sources of calories were bread and potatoes. The diet was highly monotonous. The amounts of meat, fat and

sugar were small. There was no cheese in the ration and no fresh vegetables were flown in, but vegetables were obtained from farms at the outskirts of the city and were issued, as long as the supply was available, at the rate of 2 kg. per month. The average calcium intake was estimated at less than 0.5 g. daily, a level which is much below the allowance recommended by the U. S. National Research Council (set at 1 g. per day for adults and at 1.2 to 1.4 g. for older children and adolescents), yet apparently was adequate for maintenance of health. The authors noted that only after a long search were a very few cases of mild rickets discovered in infant clinics. The intake of vitamin C from natural foods was very low (around 10 mg. per day or less) compared to the recommended allowances, but no valid conclusions can be drawn from the fact that no clinical signs of scurvy were seen, as ascorbic acid tablets were available in an appreciable though variable amount to a large part of the population.

Of interest is the design of the observations. Contrary to the procedure used in many American surveys made in Western Germany, where hundreds of thousands of individuals were examined, necessarily very superficially, the British authors selected a sample from two sections of Berlin, considered representative of the population, for a detailed study. The initial sample, examined in November 1948, consisted of 600 individuals. Of these, 366 were seen during four successive examinations. The sample was supplemented by persons of the age, sex, social status, or occupation corresponding to the characteristics of the absentees. The retention of more than 50 per cent of the initial sample must be considered a success. More detailed information about setting up the sample would have been useful to other workers.

The British observers felt that a significant part of the population was definitely undernourished as a result of the blockade. Unfortunately, no control (preblockade or prewar) data were available, or at least were not utilized in the report, and no systematic observations were made during the first visit (September 1948). The clinical evaluation of the nutritional status followed the procedure outlined by E. W. Adcock, W. H. Hammond, and H. E. Magee (*J. Hyg.* **45,** *65 (1947)*). Attention was paid to the general appearance, pallor, tired expression, lethargy, poor posture, diminished muscular tone and development, lack of luster of the hair and diminished fat. The frequency of the individual criteria was, unfortunately, not tabulated and the nutritional state was classified qualitatively as "good," "fair" and "poor." The frequency of these ratings in the sample of individuals seen in November 1948 (some five months after the start of the blockade), March 1949 (four months of somewhat improved diet), and October 1949 (four months of an almost unrestricted intake of energy-yielding foods) was given separately for men and women, and for boys and girls, without indicating the number of individuals in each of the four subgroups.

The study, as a whole, suffers from the lack of adequate standards of "good nutritional status" in the population examined, such as data on the thickness of subcutaneous tissue in well-fed, healthy individuals, and from lack of information on the incidence of signs and symptoms of "malnutrition" in the preblockade (or prewar) times. A need for cautious interpretation of some of these signs (bleeding gums, tenderness of bones, myotactic irritability) was demonstrated as a result of therapeutic trials which were negative as far as specific nutrients (ascorbic acid, vitamin D, vitamins of the B-complex) are concerned.

BLOOD PRESSURE IN UNDERNOURISHED EUROPEANS IN THE TROPICS

The internment of a group of male Europeans after the disaster of Singapore in February 1942 gave rise to interesting observations which have only recently been published (J. P. Carlile, W. R. Duff, and R. G. S. MacGregor, *J. Trop. Med. Hyg.* **55,** *121 (1952)*). A large number of men, hitherto normally accustomed to a high intake of protein and unlimited calories, were suddenly reduced to a low caloric ration, consisting almost exclusively of rice and vegetables and consequently of low protein content, particularly as regards animal protein. Considerable weight was lost by these subjects. In some cases the loss amounted to 40 or 50 per cent of the original body weight. By reinforcing the diet with limited quantities of rice polishings, red-palm oil, or beans when these could be obtained, and spreading these supplements over as long a period as possible, the more obvious vitamin deficiencies could be avoided.

A group of 150, representing a cross-section of internees from the points of view of age and physique and free of any individual who could be regarded as ill or unfit, was followed from 1942 to 1945. Because the diet of this relatively large group can be considered as somewhat similar to the low calorie, low protein rice diet advocated by Kempner and others in the treatment of hypertension (*Nutrition Reviews* **9,** *70 (1951)*), and because the treatment was perforce adhered to for a long period, the report on blood pressure in these individuals assumes particular clinical significance.

The group followed by Carlile and his colleagues was fairly heterogeneous. The age of the subjects varied between 25 and 60, their initial weights between 112 and 188 pounds, their physical condition between those accustomed to vigorous exercise and those accustomed to sedentary life. Total calories remained approximately within a range of 200 calories more or less than 2000 calories per day until 1945, when it dropped to 1500 and eventually to 1200 calories per day. Protein intake first maintained at about 50 g. per day similarly fell to 30 g. per day during 1945. Animal protein intake fell from 20 g. per day to below 10 g. per day in 1943 and never rose afterward. Fats were extremely limited, as were minerals and salt. The vitamin supply was maintained above the minimum requirement level except occasionally. Alcohol and tobacco, to which many of the subjects had been used, were, naturally, totally eliminated. Blood pressure was measured under relaxed conditions with the same instrument used throughout and cross checks were made by the two observers. The auscultatory method was employed. About 30 observations per subject were obtained in 1942, 6 in 1943, 1 in 1944 and 2 in 1945. The results were analyzed statistically, with special attention being given to age, weight loss, mental stress, and initial levels of blood pressure.

Comparison of blood pressure changes in age groups together with the average weight alterations in the same group in the four years showed that all age groups reacted in a similar fashion as far as systolic and diastolic pressures were concerned. In fact, no significant difference could be found between these various age groups by statistical analysis. Similarly, no correlation could be found between amount of weight loss and type of alterations of blood pressure levels. Changes in conditions of internment bringing about greatly increased conditions of tension and insecurity were not accompanied by concomitant rises in blood pressure.

While the initial blood pressures of the group were generally lower than those expected for a normal European group, particular attention was given to the subjects with average systolic pressures over 120 mm. They represented about 20 per cent of the sample. Surprisingly, diastolic and systolic pressures in this group rose from the onset of the internment. Systolic pressures

in particular rose for about eighteen months. After this period it fell again, but by the end of the internment period, in spite of the fact that the average body weight of the group had decreased from 176 to 131 pounds, the systolic pressure average was still definitely above the initial value.

The authors refrain from giving any interpretation of their data. While the "experiment" described by Carlile *et al.* was obviously not conducted under ideal conditions of measurement, particularly as regards sodium intake, it was unusually prolonged and free of infraction. The evidence thus obtained shows that prolonged drastic restriction of dietary calories and protein, with severe weight loss; and moderate restriction of sodium, did not result in drop of blood pressure in these individuals. It may be recalled (*Nutrition Reviews, loc. cit.*) that even in frankly hypertensive patients, only a fraction (on the order of one-third) show a clear-cut reduction in blood pressure attributable to the rice diet, most importantly to its low sodium content. The relation of diet to hypertension, while it unquestionably can be demonstrated in some cases, is still far from being understood.

DIET DURING PREGNANCY

The nutrition of the pregnant woman has been the object of several studies recently carried out in different parts of the country as well as abroad (*Nutrition Reviews* **4,** *175 (1946)*; **6,** *299 (1948)*; **7,** *139 (1949)*). The emphasis in these studies has been upon comparison of the nutrient intake of these women with the established standards as a point of reference without as detailed information concerning the dietary patterns of the subjects studied in terms of foods. Adequate nutrition is possible in diets that are made up of quite different combinations of foods, and even in the United States there is considerable variation in the dietary pattern between regional population groups.

Pointing out that the majority of studies of nutrition in pregnancy have been carried out among indigent or nearly indigent women in large urban areas, P. C. Jeans, M. B. Smith, and G. Stearns (*J. Am. Dietet. Assn.* **28,** *27 (1952)*) have studied the dietary habits of 404 pregnant women of low income groups in a rural section (Iowa). Most of these women were seen first during the third trimester of their pregnancy. Sixty-two subjects were wives of students. They received dietary instruction early in their pregnancies and served as a control group. The second group of 66 women were wives of farm laborers who had access to farm and dairy products. The largest group of 228 women were wives of town laborers. Twenty wives of unemployed laborers and 28 unmarried women were also studied. The dietary information was obtained by interviews, all of which were conducted by the same dietitian. Summary records were prepared which were then analyzed statistically in terms of both nutrients and food groups.

Analysis of the caloric intakes revealed ample levels for all groups according to the Recommended Daily Allowances of the National Research Council for the latter half of pregnancy. The protein intake was 60 g. per day or better in all but the group of wives of unemployed laborers. The average for the control group was 75.3 g. per day, as compared with the allowance of 85 g. Adequate or satisfactory intakes of thiamine and iron were reported and attributed to the universal use of enriched bread as well as to the consumption of large amounts of potatoes. This latter vegetable also contributed between 31 and 36 per cent of the ascorbic acid intake of the four indigent groups. Citrus fruits were consumed in significant amounts only by the wives of the students. Although the intakes of ascor-

bic acid for these four groups were far below the recommended allowance, only 6 of the 404 women had intakes less than 40 mg. per day; these few were not potato eaters. Vitamin A was provided by fortified margarine, fruits, and vegetables, especially tomatoes and carrots. Little was obtained in the form of milk and eggs by the indigent groups. The lack of milk in the diets of these women is also reflected in the relatively poor intakes of both riboflavin and calcium. The town laborers' wives, the wives of the unemployed, and the unmarried women averaged 0.53, 0.38, and 0.56 g. per day respectively. The farm wives received only half the recommended allowance of 1.5 g. per day. The poor milk intake was attributed partly to its expense and partly to poor refrigeration or distaste for milk on the part of the farm wives.

When all of the women in this study were divided into five groups on the basis of their protein intake it was found that some individuals from each study group were represented. It was also interesting that the relative positions of these groups with respect to all the other nutrients studied were the same as those for protein. In other words, those women who had low protein intakes were on the average among those with the lowest intakes of the other nutrients as well.

The dietary pattern that was common to all four of the indigent groups, and is probably representative of the rural indigent population of the Midwest, showed great dependence upon potatoes and cereals which were almost without exception enriched. Potato consumptions of from 10 to 20 servings weekly were common. Bread, pastries, sweet rolls, cakes and cooked cereals for breakfast in families with children were the common cereal sources. The lack of citrus fruits has already been mentioned. The variety of vegetables in the diets was quite limited. Tomatoes were used in large amounts by all the families and carrots were the most popular vegetable. Home-canned green beans, purchased canned corn and peas, cabbage as cole slaw, and leaf lettuce in season round out the assortment. The greatest deficiency in the dietary pattern of this group is the low milk intake. The contribution of enriched bread toward making these diets adequate in thiamine, iron and riboflavin immediately suggested to the authors that the inclusion of as much nonfat dry milk solids in bread as is consistent with the production of a good loaf would greatly improve the diets of these pregnant women. The cost of milk might be met by better budgeting.

This is indeed an interesting study since it depicts quite clearly the dietary pattern of an indigent rural population.

ANTIBIOTICS AND RUMEN DIGESTION

Cellulose or fiber digestion by ruminants is generally believed to be accomplished by naturally occurring microorganisms in the rumen. Many such organisms are types that would be sensitive to destruction by antibiotics or resistant to such agents. The use of a number of antibiotics should therefore serve to differentiate between organisms that are essential to the cellulytic process and those that are not essential and possibly actually inhibitory to cellulose breakdown.

R. H. Wasserman, C. W. Duncan, E. S. Churchill, and C. F. Huffman (*J. Dairy Sci.* **35,** *571 (1952)*) have recently conducted studies to determine the influence of several antibiotics on the *in vitro* digestion of cellulose. The rumen contents of a fistulated Holstein cow maintained on a normal hay and grain ration served as the medium for testing the effects of the antibiotics.

The antibiotics employed were penicillin-G (crystalline sodium salt), streptomycin (calcium chloride complex), neomycin sulfate, and synthetic chloromycetin. Each of these

was tested at several concentrations in the incubation medium.

Along with the rumen contents (200 g. per sample), the incubation medium contained weighed amounts (approximately 4 g. per sample) of cellulose in the form of filter paper. The incubation container, which consisted of a semipermeable sac, was immersed in a salt solution to facilitate dialyzing metabolic products from the system. The incubations were carried out for twenty-four-hour periods at 40° C. in an atmosphere of oxygen-free carbon dioxide and at a pH maintained between 6.2 and 6.8.

Low concentrations of penicillin in the medium (5 or 7.5 units per milliliter) stimulated the digestion of cellulose. These levels of penicillin in the medium increased cellulose digestion 15 to 20 per cent above the values obtained without the antibiotic. A higher concentration of penicillin (15 units per milliliter) resulted in a 35 per cent decrease in cellulose digestion when compared with control values.

Streptomycin had no influence when present in the medium at a level of 12.5 micrograms per milliliter, but decreased cellulose digestion 15 to 20 per cent below control values when present in the medium at concentrations of 50 or 25 micrograms per milliliter.

Chloromycetin, when present in the medium at a level of 25 micrograms per milliliter, decreased cellulose digestion 30 per cent, and twice this level of antibiotic lowered cellulose digestion to less than 50 per cent of control values.

Neomycin stimulated cellulose digestion at all levels tested, namely 6.25, 15.5, and 25 micrograms per milliliter of medium. The lowest concentration gave the greatest stimulation, an increase in cellulose digestion of 30 per cent above control values. The mediums were also periodically assayed during the incubation in order to determine the ability of the antibiotics to withstand the action of the rumen contents. Approximately 40 per cent of the neomycin disappeared during the first forty-five minutes of incubation and thereafter loss of this antibiotic was slight.

Penicillin decreased to less than 25 per cent of that added to the medium during the first forty-five minutes and then remained at a constant level. Chloromycetin disappeared rapidly from the medium and could not be detected after four hours of incubation.

Streptomycin increased above the initial values during the first eight hours of incubation and remained at a high level throughout the twenty-four-hour incubation period.

The observed changes in cellulose digestion by rumen contents as a result of including antibiotics in the mediums are interpreted as the result of changes in the microbial populations that attack cellulose. This is in line with the belief expressed by others that certain antibiotics are growth stimulators for animals because they rid the intestinal tract of undesirable microbial forms and permit desirable forms to increase in number (*Nutrition Reviews* **8,** *298 (1950)*; **10,** *107, 211 (1952)*).

The above study by Wasserman and associates did not include aureomycin. It should be mentioned that this antibiotic was reported to decrease fiber digestion in steers (M. C. Bell, C. K. Whitehair, and W. D. Gallup, *J. Animal Sci.* **9,** *647 (1950)*).

Studies of the type reported by these workers deserve the attention of nutritionists. The technic employed should be useful in answering many questions relating to rumen microorganisms and their influence on the nutrition of the host (*Nutrition Reviews* **9,** *59 (1951)*).

FLUORINE IN CATTLE RATIONS

In recent years, both in this country and in Europe, there have been reports of damage to cattle as the result of the emission of fluorine compounds into the air from industrial operations. These compounds settle on, or are absorbed by, herbage that may be eaten by livestock. Damaging effects are also known to occur when cattle are fed mineral supplements containing rock phosphate which may in turn contain as much as 3.5 per cent of fluorine.

Field investigations over recent years have pointed to a need for precise methods for the diagnosis of fluorosis in cattle. Many of the symptoms, such as lameness, dry skin, elongated hooves, diarrhea, impaired appetite and emaciation, are not symptoms unique to fluorosis. A need is also indicated for more accurate estimates of the borderline between damaging and nondamaging intakes of fluorine over extended periods in cattle.

W. E. Rand and H. J. Schmidt (*Am. J. Vet. Res.* **13,** *50 (1952)*) have recently reported observations relative to the problem of fluorine damage in cattle. The studies involved analyses for fluorine in the drinking water, in the feed, and in the bones and urine of cattle on several ranches in Arizona. Fluorine intake levels were compared with bone levels, excretion levels, and also with the extent of visible symptoms attributable to fluorine poisoning.

Damage to teeth, characterized by ineradicable mottling and abnormal wearing of the incisors, was the first and most delicate measure of abnormal or excessive fluorine intake in cattle. However, the symptoms that are generally first noted by the ranchers are lameness and stiffness, with some palpable exostoses on the long bones, lower jaws and ribs. These symptoms actually occur considerably later than the observed earlier damage to the teeth. It was observed, nevertheless, that many of the cattle in the study had mottled and abnormally worn teeth, but that this condition in no detectable way detracted from the economic value of the animals.

The results of the Arizona study indicated that the fluorine content of urine was well above 10 parts per million (p.p.m.) when obvious damage from fluorine ingestion could be detected. From an extensive review of the literature on fluorosis in cattle (H. J. Schmidt and W. E. Rand, *Am. J. Vet. Res.* **13,** *38 (1952)*) it was concluded that 10 p.p.m. of fluorine in the urine represents the borderline between damaging and nondamaging intakes.

Cattle on a fluorine intake sufficient to excrete 10 p.p.m. of the element in the urine usually stored 4000 p.p.m. or more of fluorine in the bones (dry, fat-free basis). Bone values up to 8000 p.p.m. were found in cattle that were ingesting 2 mg. of fluorine per kilogram of body weight per day for a period of five years. Some of this excess bone-stored fluorine continued to be eliminated in the urine for eight months after the animals were returned to rations of low fluorine content. Urine levels of fluorine thus are a safe measure of level of fluorine ingestion only if the previous history of fluorine intake is known.

The Arizona studies were conducted on ranches where the drinking water contained as much as 18 p.p.m. of fluorine. Green forage analyses indicated 15 to 25 p.p.m. fluorine content, and hay samples ranged from 1 to 6 p.p.m.

It should be recalled that the drinking water of most communities in the United States contains less than 1.0 p.p.m. of fluorine, in many cases as low as 0.1 p.p.m. A number of cities are now adding 1.0 p.p.m. of fluorine to the drinking water. It has been found that this amount is beneficial to the teeth of children, reducing the incidence of tooth decay. Waters containing 2.0 p.p.m. or more of fluorine will cause mottling of teeth in children.

The studies with cattle reported by Rand and Schmidt confirm earlier studies in that the cattle consuming as high as 1.0 mg. of fluorine per kilogram body weight per day over periods of five or ten years showed no detectable effects except slight mottling and wear on the incisor teeth. The fluorine level in the diet of 2.0 mg. per kilogram body weight per day produced severe damage to teeth and bones, and resulted in lameness, stiffness, emaciation and general unthriftiness. In general, it has been known from earlier studies that cattle can apparently tolerate five to ten times the dietary level of fluorine that is considered harmful to humans.

The Arizona study is disappointing in that it has not contributed much toward more precisely defining damaging versus nondamaging levels of fluorine in cattle feeds. The actual intake levels were generally calculated from estimated intakes of forage and water by cattle under range conditions. In order to determine more precisely the relation between fluorine intake, bone storage, and excretion levels, carefully controlled feeding experiments are needed.

EFFECTS OF SPRAYS AND STORAGE CONDITIONS ON POTATOES

The increasing use of sprays in the production of agricultural crops necessitates continual alertness toward possible side effects incidental to the main objectives of the spray program. Profound physiologic effects are brought about in plant life by relatively minute amounts of certain chemical compounds absorbed through the leaves or roots. The more obvious effects are first noted, but many more obscure or slower effects are no doubt often overlooked. The situation may sometimes become very confusing if the substance starts some synthetic process in the plant which later affects the physiologic processes of animals that used the plant as their food. Such effects could have far-reaching consequences. Nontoxicity of a spray material as such does not necessarily mean that plants treated with it may not later themselves become toxic to animal or human life.

Maleic hydrazide has been intensively investigated recently because of its remarkable property of stopping cell division in plants. A natural application would appear to be the inhibition of sprouting in harvested potato tubers. The question was investigated for sprays on leaves before harvest and treatment of tubers after harvest by D. R. Paterson, S. H. Wittwer, L. E. Weller, and H. M. Sell (*Plant Physiol.* **27,** *135 (1952)*). Irish Cobbler and Pontiac varieties were sprayed with 500, 1000 and 2500 parts per million (p.p.m.) at different times shortly before harvest. Comparisons were made with controls, with 2,4,5-trichlorphenoxyacetic acid applied to the foliage, and with a dust formulation of the methyl ester of alpha-naphthaleneacetic acid applied to the harvested tubers. No tuber injury was evident nor did any reduction in yield, size, or quality result from any of the treatments. The treatment at 1000 p.p.m. maleic hydrazide was quite effective in the induction of dormancy. This and often the 500 p.p.m. level compared favorably with the comparison spray treatment. The latest applications to foliage were not so effective as earlier ones. Earlier applications of 2500 p.p.m. reduced sprouts after seven months storage almost to zero. Treatments were more effective at a storage temperature of 45° F. than at 55° F.

Maleic hydrazide is unique among sprays in that it apparently causes no abnormalities or loss of quality of potatoes, even at concentrations which induce permanent dormancy. It completely inhibits growth of sprouts at the apical end of the tuber. These results are favorable for another reason,

namely, that the higher storage temperatures (above 45° F.) at which maleic hydrazide is effective are conducive to less pronounced degradation of starch to produce sweetened potatoes of inferior quality. It was shown that the foliar spray caused reduction in both reducing and nonreducing sugar contents in potatoes stored seven months. This was especially effective at 45° F. Also, potato chips processed from treated and stored tubers had better color quality than those from untreated stored tubers. Other data indicate that maleic hydrazide is a selective respiratory inhibitor.

Nutritional values of such tubers from sprayed plants should be evaluated before widespread use is made of such sprays on food crops.

The effects of storage of potatoes in nitrogen, air and pure oxygen on ascorbic acid content are reported by J. Barker and L. W. Matson (*New Phytologist* **51,** *90 (1952)*). Tubers were placed in sealed jars through which the gas was passed at a rate of 2 liters per hour. Temperatures studied were 10, 25, and −0.6° C. By exclusion of oxygen, the content of ascorbic acid was almost completely stabilized, apparently due to a direct effect on the processes involved in the loss of this vitamin. A major side-effect of the exclusion of oxygen was to alter the balance between sucrose and hexose sugars toward an increased proportion of hexose sugars. This may illustrate an impairment of power to synthesize sucrose.

DIET AND X-IRRADIATION

Evidence has been presented recently by B. H. Ershoff (*J. Nutrition* **47,** *289 (1952)*) to indicate that liver contains a factor or factors that protect female rats from the damaging action of repeated small doses of x-rays.

The basal diet used consisted of the following (in per cent): sucrose, 61; vitamin-test casein, 24; salt mixture, 5; and cottonseed oil, 10. It contained the following synthetic vitamins (milligrams per kilogram of diet): thiamine hydrochloride, riboflavin and pyridoxine hydrochloride, 20 each; calcium pantothenate and niacin, 60 each; ascorbic acid, 200; 2-methyl-naphthoquinone, 10; and choline chloride, 2000. Vitamins A and D were added at the rate of 4000 and 400 U.S.P. units respectively per kilogram of diet. In addition, each rat received 4.5 mg. of alpha-tocopherol acetate once a week. A high-vitamin diet consisted of the basal diet with the vitamins supplied in double the normal amount and fortified further with the following (milligrams per kilogram of diet): biotin, 5; folacin, 10; para-aminobenzoic acid, 400; inositol, 800; and vitamin B_{12}, 0.15. A liver diet consisted of the basal diet except that 10 per cent of the sucrose was replaced with an equal amount of dried whole liver powder.

In the first experiment, weanling rats were fed the diets for twenty-two weeks and then were exposed to 200 r of x-irradiation once each week for a period of eight weeks (total exposure per rat equal to 1600 r).

With this treatment 60 per cent of the rats on the basal diet and 70 per cent of those on the high-vitamin diet died from x-ray damage before the first fatality occurred among the rats receiving the liver diet. The liver diet thus gave significantly better protection against x-irradiation than did the diet fortified with normal or high amounts of pure vitamins. Thirty days after the last x-ray dose, the average survivals were 20, 40, and 80 per cent respectively for the rats on the high-vitamin, the basal, and the liver diet. Fatalities occurred thirty-five to forty days after the first x-ray dose among the rats receiving the basal and the high-vitamin diets, and after an average of

sixty-one days in those receiving the liver diet.

No significant change in body weight or gross appearance was observed under any of the dietary treatments as a result of the x-ray dose used.

In the second experiment the same diets were fed. After sixteen weeks 20 weanling rats on each diet were given a single x-ray dose of 800 r, whereas an equal number on each diet received a single dose of 850 r. The single heavy doses of x-ray completely overcame the protective effects of the liver diet noted in the first experiment. The mortality rate was between 55 and 60 per cent for all groups receiving 800 r of x-ray, and between 70 and 90 per cent for those receiving the 850 r dose. Surviving animals on all diets lost about 10 per cent of their body weight during the thirty-day period following irradiation.

The third experiment was designed to study the effects of x-ray on growth and survival. The same diets were employed and, as in the first experiment, the x-ray dose consisted of 200 r per rat per week for eight weeks. The irradiations were begun on the fourth day and 10 rats, or one half of the total on each diet, served as controls and were not irradiated. Under these conditions x-irradiation significantly retarded growth rate in all groups. Those not receiving x-ray gained between 186 and 203 g. in one hundred days whereas the x-ray-treated rats gained between 104 and 151 g. in a similar period. The liver diet gave somewhat better growth in both the x-ray-treated and the nontreated rats than did the basal or the high-vitamin diets. All treated rats on the basal diet died in an average of sixty-eight days after the first x-ray dose. Ninety per cent of those on the high-vitamin diet succumbed in sixty-six days, whereas 70 per cent of those on the liver diet died, with an average survival period of one hundred and seventeen days.

These studies clearly demonstrated that the damaging action of sublethal doses of x-irradiation could be at least partially overcome by fortifying a purified diet containing synthetic vitamins with whole liver powder. The protective effect of liver under these conditions apparently was not caused by any of the known vitamins which it might contain since liberal additions of these were without effect. Whether or not still unidentified factors are involved is not known. It should be pointed out that the substitution of 10 per cent of sucrose by an equal amount of whole liver, as was done in these experiments, resulted in a considerable increase in the protein level of the diet, a factor which was not mentioned, but which may be important.

Earlier reports from the same laboratories have ascribed special protective properties to liver in the diet under other stress conditions. Liver was reported to counteract the toxic effect of thyroid (B. H. Ershoff, *Proc. Soc. Exp. Biol. Med.* **64,** *500 (1947)*; of promin (Ershoff and H. B. McWilliams, *J. Am. Pharm. Assn.* **38,** *274 (1949)*; and of atabrine (Ershoff, *J. Nutrition* **35,** *269 (1948)*).

It is possible that the stress occasioned by x-ray treatment may simulate the toxic reactions of such drugs and that the factors involved in protecting the animals are the same. Since dietary liver was nonprotective when large doses of x-ray were given, it is likely that the protective effect obtained with liver under lighter x-ray dosage represents a counteraction of toxic substances that accumulate under the chronic stress condition of sublethal x-ray treatment.

PROTEIN METABOLISM IN DIABETES

One of the chief symptoms of severe untreated diabetes is generalized wasting of the body, an indication that proteins as well as fats are sacrificed. Whether this protein loss is a direct consequence of the lack of insulin, or is due to secondary effects of the diabetes such as ketosis, malnutrition, or renal failure, has long been a controversial question among clinicians (*see* J. P. Peters and D. D. Van Slyke, "Quantitative Clinical Chemistry," *p. 685, 2d edition, Williams and Wilkins, Baltimore (1946)*).

Experimental results were not unequivocal. The fact that insulin induces a fall in the concentration of amino acids in the blood of the normal dog (W. D. Lotspeich, *J. Biol. Chem.* **179,** *175 (1949)*), the eviscerated dog (I. A. Mirsky, *Am. J. Physiol.* **124,** *569 (1938)*), and the rat (E. G. Frame and J. A. Russell, *Endocrinology* **39,** *420 (1946)*) loses some of its significance when the normal concentration of amino acids in plasma is contrasted with the much greater amounts of nonprotein nitrogen in tissues (F. Friedberg and D. M. Greenberg, *J. Biol. Chem.* **168,** *411 (1947)*).

New evidence has now been obtained by workers at the Universities of California and Yale which contributes a much clearer basis for our understanding of the effect of diabetes on protein metabolism. I. L. Chaikoff and L. L. Forker (*Endocrinology* **46,** *319 (1950)*) examined the effect of insulin administration on the nitrogen excretion of depancreatized dogs. Every precaution was taken by these workers at the University of California to establish ideal "balance study" conditions and to eliminate possible short-range effects from operative trauma or sudden changes in insulin dosage. The experimental animals were maintained on weighed high protein rations of constant composition. Nitrogen excretion was determined daily. Doses of insulin varying between 0 and 16 units per day were administered for periods of at least one month, with injections given every twelve hours. Under these experimental conditions, a linear relation was obtained between insulin dose and nitrogen retention. Nitrogen equilibrium corresponded to 8 units per day; negative balance was obtained as the dose was decreased; 16 units led to a positive balance. Changes in nitrogen excretion followed changes in insulin dosage within the next twenty-four hours.

The loss of body protein in the absence of insulin can in turn be due either to a decreased rate of protein synthesis or to an increased rate of protein catabolism. Following two independent lines of investigation, H. D. Hoberman (*Yale J. Biol. Med.* **22,** *341 (1949)*) and L. L. Forker and his collaborators (*J. Biol. Chem.* **188,** *31, 37 (1951)*; **196,** *829 (1952)*) showed that diabetes affects the rate of protein synthesis rather than protein breakdown.

Hoberman (*loc. cit.*) followed the urinary excretion of heavy nitrogen administered as labeled glycine to diabetic rats. The experimental animals were young adult males alloxan-treated and fasted for twenty-four hours before the administration of isotopic glycine. The urine was collected daily, its volume being increased by repeatedly injecting the animals with saline, and its isotope content was determined.

By making the assumptions that amino acids and proteins constitute two separate homogeneous pools of nitrogen and that the rate at which nitrogen moves out of either pool is proportional to its concentration in that pool, it became possible to calculate the average rate of synthesis of proteins from amino acids and the average rate of amino acid catabolism. By this method—the theoretic foundation of which is admittedly open to criticism—Hoberman was able to conclude that in alloxan-diabetes there is both depression of protein synthesis from amino acids and acceleration of protein breakdown. Since it was found (*loc. cit.*)

that ACTH administration also accelerated protein breakdown, Hoberman also studied adrenalectomized alloxan-diabetic animals. In these animals there is no acceleration of protein breakdown, and the rate of amino acid catabolism is reduced to that presented by adrenalectomized nondiabetic animals. On the other hand, the rate of protein synthesis from amino acids remains depressed. Hoberman concluded that this decreased rate of synthesis is therefore the specific effect of absence of insulin on protein metabolism.

The necessity of unsupported postulates for the interpretation of experimental data was avoided by L. L. Forker, I. L. Chaikoff, C. Entenman, and H. Tarver (*J. Biol. Chem.* **188,** *37 (1951)*) in their study of the influence of diabetes on the synthesis of protein. They compared the rates of formation of muscle protein in normal and depancreatized dogs by following the incorporation of labeled methionine (containing radioactive sulfur). In a previous publication (Forker, Chaikoff, Entenman, and Tarver, *Ibid.* **188,** *31 (1951)*) it was shown that, while oxidation of S^{35} to sulfate occurred readily in the nephrectomized animal, it did not take place in the eviscerated animal. This finding suggested that the liver was the chief seat of oxidation. It had also been shown (F. Friedberg, Tarver, and D. M. Greenberg, *Ibid.* **173,** *355 (1948)*) that when radioactive methionine was administered to dogs it was rapidly incorporated into tissue proteins and the radioactive sulfur was only slowly released from the various organs. The pattern of incorporation of S^{35} in order of decreasing activity was as follows: intestinal mucosa, pancreas, spleen, kidney, plasma, liver, testes, heart, brain and muscle.

These two groups of findings were used to best advantage by Forker and his collaborators. By nephrectomizing their animals they prevented excretion of S^{35}, and thus avoided an important difference between normal and diabetic animals, namely, the high urinary loss of the latter. By eviscerating their animals, they eliminated the organs and tissues most active in incorporating methionine into protein, or oxidizing sulfur into sulfates. Comparable concentrations of labeled amino acids could then be achieved in the diabetic and the normal animals, with incorporation into muscle protein the almost exclusive fate for methionine. Under these experimental conditions the normal animals incorporated S^{35} into muscle proteins three to five times more rapidly than the diabetic dogs. This difference was eliminated if the diabetic animals were treated with insulin. Thus it was again demonstrated that diabetes depresses the rate of protein synthesis.

Finally, L. L. Forker and I. L. Chaikoff (*J. Biol. Chem.* **196,** *829 (1952)*) examined the rate of protein catabolism in normal and depancreatized dogs by following the decrease in specific activity of the circulating serum proteins of animals injected with S^{35}-labeled serum protein. The latter was prepared by administering radioactive methionine to a dog, exsanguinating the animal thirty hours later, dialyzing and drying the serum, and dissolving the preparation in physiologic saline. These labeled proteins were then injected intravenously into depancreatized dogs in which the diabetes was either carefully controlled with insulin, or acute because of insufficient insulin dosage. The disappearance of the labeled protein was followed for about two weeks. Forker and Chaikoff found that serum protein continued to be degraded at the normal rate within the accuracy of measurement, whether the diabetes was controlled or not. Furthermore, a normal rate of disappearance was found to prevail for the radioactive sulfur of the albumin and of the globulin fractions.

From these reports it appears probable that the main effect of diabetes on protein metabolism is to decrease the rate of muscle protein synthesis. This tissue contains the bulk of body protein, and the decrease in

formation rate of the order of 60 to 80 per cent observed by Forker and his collaborators in diabetic animals is sufficient to account for the loss of body protein in diabetes.

While much remains to be explained concerning the mechanism of this decrease in protein synthesis, the reports of Hoberman, and especially those of Chaikoff and his co-workers, represent essential contributions to our understanding of metabolism in diabetes.

ASCORBIC ACID AS A COENZYME

Over ten years ago it was shown by R. R. Sealock and H. E. Silberstein (*Science* **90,** *517 (1939)*; *J. Biol. Chem.* **135,** *251 (1940)*) that intermediary products of tyrosine degradation could be found in the urine of scorbutic guinea pigs when large doses of the amino acid were administered to the animals. Considerable interest was created by this finding, which seemed to point the way both to knowledge of various steps in the catabolism of tyrosine and to discovery of an enzymatic role for vitamin C.

Recently, a number of reports have appeared on the mechanism of oxidation of L-tyrosine (*Nutrition Reviews* **10,** *117, 212 (1952)*). In particular, B. N. La Du and D. M. Greenberg (*J. Biol. Chem.* **190,** *245 (1951)*) demonstrated the existence, in the liver, of an enzymatic system which degrades tyrosine by transamination to para-hydroxyphenylpyruvic acid, decarboxylation yielding homogentisic acid, an opening of the benzene ring and eventual oxidation of the products thus formed. These results are substantially in agreement with those of other workers, B. Schepartz (*Ibid.* **193,** *293 (1951)*; *Nutrition Reviews* **10,** *117 (1952)*) in particular.

The role of ascorbic acid in the first steps of this degradation has not hitherto been completely clear. W. E. Knox and M. LeMay-Knox (*Biochem. J.* **48,** *xxii (1951)*; **49,** *686 (1951)*; *Nutrition Reviews* **10,** *92 (1952)*) have found that transamination is the limiting reaction in the degradation of tyrosine by rat liver homogenates. Addition of ascorbic acid as well as of alpha-ketoglutarate to a refined enzyme preparation increased considerably the rate of reaction. Isoascorbic acid was found to be as effective as ascorbic acid. Either seemed to be required for the continuing oxidation of para-hydroxyphenylpyruvic acid. B. N. La Du (*Fed. Proc.* **11,** *244 (1952)*; *Nutrition Reviews* **10,** *212 (1952)*) has presented evidence suggesting that the stimulatory effect of ascorbic acid was on the step following transamination, the transformation of para-hydroxyphenylpyruvic acid to 2,5-dihydroxyphenylpyruvic acid.

A more recent article (R. R. Sealock, R. L. Goodland, W. N. Sumerwell, and J. M. Brierly, *J. Biol. Chem.* **196,** *761 (1952)*) sheds additional light on the possible role of ascorbic acid as a coenzyme in tyrosine oxidation and its behavior in tissues.

Unlike Knox and Le May-Knox, who used rats, and La Du, who used rabbits, Sealock and his collaborators worked with severely scorbutic guinea pigs. They reasoned, from previous *in vivo* work, that homogenates prepared from the livers of such animals should contain little, if any, ascorbic acid and would not oxidize tyrosine. Actually, they found that these "scorbutic" homogenates oxidized the amino acid almost as completely as those from normal animals. As the amount of liver tissue incubated with 1 mg. of tyrosine increased, the amount of tyrosine oxidized by the "vitamin C-deficient" homogenates increased, up to a level 94 per cent of that corresponding to "control" homogenates. This led Sealock and his co-workers to determine the ascorbic acid content of normal and scorbutic guinea pig liver, measuring

the bound form (*Nutrition Reviews* **10,** *319 (1952)*) as well as the free form of the vitamin. While both were decreased compared with the normal values, it was found that the bound form was proportionately far less affected than free ascorbic acid. The authors reasoned that, because the hitherto underestimated bound ascorbic acid was thus in part retained even in severe scurvy, there was enough of the vitamin to oxidize tyrosine if sufficient tissue was used.

Sealock and his collaborators next prepared a liver acetone powder by a modification of the method of La Du and Greenberg (*loc. cit.*). Refined enzyme preparations were obtained by further purification. In agreement with previously mentioned reports (Knox and Le May-Knox; La Du, *loc. cit.*), they found that ascorbic acid helped in restoring the activity of the dialyzed preparation. A dissociation constant characteristic of the linkage of ascorbic acid to a hypothetic protein enzyme was calculated. The concentration of vitamin beyond which no further increase in activity took place was also measured. Besides D-isoascorbic acid, it was found that D-glucoascorbic acid and reductone, all of them compounds containing, like ascorbic acid, an enediol linkage, restored the tyrosine-oxidating activity of dialyzed extracts. Derivatives of ascorbic acid in which the enediol structure was blocked were inactive. Glutathione, an antioxidant not containing this functional group, was also inactive. This was taken by Sealock and his colleagues to mean that active compounds did not act merely to prevent the oxidative destruction of ascorbic acid.

The authors conclude from these experiments that dehydroascorbic acid, in conjunction with and bound to an apoenzyme, causes specific dehydrogenation of the tyrosine keto-acid, transferring the hydrogen to suitable acceptors. The reaction is dependent on an unblocked oxidation-reduction enediol center, with all analogous compounds containing this group potential coenzymes.

The possibility that "if this interpretation is correct, a biochemical function of vitamin C is defined" is not confirmed by the fact that in this particular reaction ascorbic acid can be replaced by glucoascorbic acid. The effects of administering this compound to experimental animals have been reviewed previously (*Nutrition Reviews* **4,** *123 (1946)*; **9,** *177 (1951)*). It may be useful to recall that, while it is toxic at high levels (5 or 10 per cent), the animals can be protected by addition of liver powder to their purified diets. Ascorbic acid is not corrective. Furthermore, microscopic criteria of scurvy are absent from the symptoms of glucoascorbic acid toxicity. In these respects, glucoascorbic acid cannot therefore be considered a true antivitamin. Furthermore, there is evidence (W. G. Shafer, *J. Dent. Res.* **29,** *831 (1950)*; *Nutrition Reviews* **9,** *177 (1951)*) that glucoascorbic acid alleviates somewhat, but only in small part, some of the signs of scurvy in guinea pigs. If the hypothesis of Sealock *et al.* on the role of ascorbic acid as a coenzyme in the oxidation of para-hydroxyphenylpyruvic acid is to be retained, one is led to conclude that this particular function is not fundamental to the etiology of scurvy. It is furthermore a type of reaction in which the chemical specificity of vitamin C is restricted to the enediol structure so that it can be replaced in this limited respect by other compounds containing the same grouping. In other reactions, fundamental to the lesions of scurvy, such replacement has no effect.

Until recently, no analogous situation was known to exist where the specificity of vitamin structure depended on the particular function considered. The work of H. Dam and his co-workers on the effect of replacing vitamin E by a number of substances on various symptoms of deficiency (*see Nutrition Reviews* **10,** *149 (1952)*) furnishes a model of the existence of such partial replacements. Whether this applies in fact to vitamin C as it does to vitamin E will await further work on the subject.

SPECIES DIFFERENCES IN IRON ABSORPTION

The influence of diet on iron absorption has been a subject of continued investigative interest. In a previous review (*Nutrition Reviews* **8,** *7 (1950)*) the observations of T. D. Kinney, D. M. Hegsted, and C. A. Finch (*J. Exp. Med.* **90,** *137, 147 (1949)*) were described. These investigators showed that large amounts of iron were absorbed by rats fed a corn grits diet supplemented with greater than physiologic amounts of iron. Much more iron had to be added to normal diets to achieve a comparable degree of body iron retention. Dietary experiments indicated that the low phosphorus content of the experimental diet was responsible for the heightened iron absorption.

In view of the known species differences in the metabolism of iron, these workers have extended their observations to other animal species. Excessive iron storage occurred in dogs on a corn grits diet supplemented with ferric citrate (C. A. Finch *et al., Blood* **5,** *983 (1950)*). Because of the experimental difficulties involved in quantitative dietary studies in dogs, detailed observations in this species were not carried out.

Recently a comparative study of iron absorption in rats, guinea pigs, chicks and mice has been done by this group of workers (D. M. Hegsted, C. A. Finch, and T. D. Kinney (*J. Exp. Med.* **96,** *115 (1952)*). As in the previous studies, a diet containing 20 per cent corn grits and 80 per cent lard plus vitamin supplements was used. It was necessary to modify the diet for guinea pigs to contain 2 per cent lard, 10 per cent cellulose and 88 per cent corn grits. The guinea pigs also received ascorbic acid by subcutaneous injection. Ferric chloride was added to these diets in concentrations of from 0.25 to 12 per cent. The results obtained with the experimental diet were compared with those obtained on a commercial dog chow diet.

It is unfortunate that the experimental diets were fed for different periods (between twenty and eighty days), because it makes interpretation of results more difficult. The absorption of iron was judged, histologically, by the deposition of iron pigments and by analyzing the liver for iron. The liver iron in all species was higher on the corn grits diet as compared with the chow diet of comparable iron intakes. There was little difference of liver iron among the species prior to iron supplementation. The levels varied from 10 to 30 mg. per 100 g. of liver. On the experimental diets the liver iron rose more rapidly in chicks and mice than in rats. A more rapid absorption of iron from the control diets also occurred in this species. The liver iron levels in guinea pigs rose less on the experimental diets than those in rats.

These experiments carried out with greater than physiologic amounts of iron in the diet are of interest. Increased iron absorption by all species on the corn grits, low phosphorus diet emphasizes the general importance of the lumina in iron absorption. Considerable evidence suggests that the intestinal mucosa can also influence the absorption of iron, depending on the needs of the body. The species differences observed in these experiments might be attributed to quantitative differences in mucosal function.

THYROXINE AND THIOURACIL ON MILK COMPOSITION

In human thyroid dysfunction the observed relation between thyroxine and carotene is made more understandable by an analogy involving animal experimentation. In rats receiving dietary carotene thyroxine accelerated repletion of liver stores of vitamin A while thiourea had the opposite effect (R. M. Johnson and C. A.

Baumann, *J. Biol. Chem.* **171,** *513 (1947)*). In rats deprived of dietary carotene thyroxine accelerated depletion of hepatic stores while thiourea had the opposite effect (B. Kelley and H. G. Day, *Ibid.* **175,** *863 (1948)*).

Recent work has shown a correlation between tocopherol intake and the carotene content of cow's milk (*Nutrition Reviews* **10,** *269 (1952)*). The effects of thyroxine on the composition of cow's and goat's milk were studied at the Hannah Dairy Research Institute in England by R. Chanda and E. C. Owen (*Biochem. J.* **51,** *404 (1952)*).

In the latter experiments 6 lactating Ayrshire cows were studied for seven dietary periods as follows: (1) carotene-free diet, (2) carotene diet, (3) carotene-free diet, (4) carotene diet with 2 cows receiving in addition 10 mg. thyroxine daily, 2 receiving 20 mg. thiouracil daily, and 2 serving as controls, (5) carotene diet, no supplements, (6) carotene-free diet, supplements as in (4), (7) carotene-free diet, no supplements. The carotene diet contained a concentrate of whole oats, beans and dried grass, while the carotene-free diet contained the same amount of digestible nutrients and protein.

Eight lactating goats were studied for three dietary periods: (1) carotene diet, (2) carotene diet with 2 goats receiving in addition 10 mg. thyroxine daily, 2 receiving 20 mg. thiouracil daily, and 2 receiving 10 mg. thyroxine and 1 mg. stilbestrol daily, (3) carotene diet. The thyroxine administered was sufficient to cause hyperthyroidism in the goats.

On alternate days fat and vitamin A were estimated in goat milk. Two goats were killed and the liver and kidney levels of carotenoids and vitamin A determined. Cow's milk from alternate evening milkings was analyzed for vitamin A and carotene. Separation of carotene, lutein, vitamin A alcohol and vitamin A ester in milk fat was accomplished by chromatography.

The analytic procedure was tested by determining recoveries of vitamin A alcohol and ester added to milk fat. Recovery ranged from 80 per cent for 5 I.U. to 93 per cent for 30 I.U. added. Cow's milk contained appreciable amounts of carotene and 6 per cent of its vitamin A as alcohol, while goat's milk contained no carotene or vitamin A alcohol. Goat's milk contained more vitamin A per gram of fat than cow's milk (39 I.U. as compared to 21 I.U.).

Cow colostrum is richer in carotene than the milk which is secreted later. Pigments of both goat liver and goat colostrum contain beta-carotene and another yellow pigment presumed to be xanthophyll or lutein. Goat kidneys contain no carotenoids or other yellow lipochromes.

All cows on the carotene-free diet showed a drop in the yield of carotene and the percentage of carotene in milk fat. On addition of dietary carotene the yield of carotene, percentage of carotene and carotene concentration in milk all increased. Again on the carotene-free diet the carotene in milk decreased to its former level.

When thyroxine or thiouracil was added during the carotene dietary period, the cows receiving thyroxine produced noticeably larger increases in the yield of carotene than did the controls, while the cows receiving thiouracil showed no increase. Evidently thyroxine facilitated the transfer of carotene to milk since the concentration of carotene in milk increased at the same time as the yield of milk increased.

When thyroxine and thiouracil were discontinued during the carotene dietary period, the controls showed a constant concentration of carotene in milk while the cows previously receiving thyroxine had a marked diminution in carotene concentration. By contrast, cows previously receiving thiouracil now showed increased carotene yield and percentage of carotene in both milk and fat. Neither thyroxine nor thiouracil affected the carotene content of milk when the cows received carotene-free diets.

In cows receiving carotene diets, discontinuance of thiouracil produced an effect similar to adding thyroxine. Both procedures

caused an increase of vitamin A in the milk. Conversely, discontinuance of thyroxine produced results like the addition of thiouracil: both caused a decrease in vitamin A in milk.

When carotene was absent from the diet, the proportion of vitamin A in alcohol form increased. When carotene was added, reversion to a normal ratio occurred. Thyroxine given to animals on carotene-free diets caused a large increase in the proportion of vitamin A present as alcohol.

In the experiments on goat's milk, analyses confirmed the observation that carotenoids were present only in minute amounts. The vitamin A was found to be present only in the esterified form. Beta-carotene was found in the post partum mammary secretion, indicating that neither thyroxine nor estrogens influence its appearance. Otherwise the effects in goat's milk were similar to those in cow's milk.

The results of these experiments may be explained by assuming that dietary carotene on absorption is transformed into the ester form of vitamin A (S. Y. Thompson *et al.*, *Brit. J. Nutrition* **4,** *398* (*1950*)). Then it passes via the lymph, the thoracic duct, and blood stream to the mammary gland. In the cow some carotene probably accompanies the vitamin A ester since both are constituents of the emulsified fat in the blood.

Most of the vitamin A in the blood is thought to be in the alcohol form. J. Glover, T. W. Goodwin, and R. A. Morton (*Biochem. J.* **41,** *97* (*1947*)) indicate that reserves of vitamin A are present in the liver as the ester, but are in equilibrium with vitamin A alcohol in the blood. Thus if a cow is deprived of carotene and thereby forced to mobilize its hepatic reserves, an increase in vitamin A alcohol in the milk would be expected. This has been found in these experiments.

J. D. Greaves and C. L. A. Schmidt (*Am. J. Physiol.* **116,** *456* (*1936*)) have shown that thyroxine accelerates the release of vitamin A reserves from livers of rats on vitamin A-deficient diets. This explains the effect of thyroxine in causing increased vitamin A alcohol in milk when cows were deprived of carotene. Kelley and Day (*loc. cit.*) found that thiouracil retarded the rate of depletion of hepatic stores of vitamin A in rats on a vitamin A-deficient diet. In cows on a carotene-free diet thiouracil caused a decrease in vitamin A in milk.

In rats Johnson and Baumann (*loc. cit.*) showed that thyroxine increased hepatic storage of vitamin A from dietary carotene, but not from preformed dietary vitamin A. Thiourea inhibited storage from dietary carotene, but not from dietary vitamin A. Thus in the cow the effect of thyroxine in stimulating the apparent digestibility of carotene is possibly due to stimulation by the hormone of conversion of carotene to vitamin A in the intestinal mucosa. Thyroxine does not increase absorption of carotene as such. The authors assume in their discussion that once carotene has passed into the lymph from the intestine it no longer serves as a source of vitamin A to the cow. When no carotene is fed, thyroxine causes no change in the output of milk carotene.

Thyroxine causes an animal deprived of carotene to mobilize vitamin A alcohol from its liver stores and increase the blood level, while thiouracil inhibits this reaction. Thyroxine increases the rate of transfer to milk of carotene and vitamin A when an animal is fed carotene, whereas thiouracil has the converse effect. Thus this work substantiates other observations in animal experimentation and human illness involving thyroid dysfunction. Valuable information is presented on effects produced in milk carotene and vitamin A levels by thyroxine and thiouracil.

THE EFFECT OF ETHIONINE ON PANCREAS AND LIVER ENZYMES

Ethionine is a methionine analogue in which an ethyl group is substituted for the methyl group. The effect of D-L ethionine administration has been reported by E. Farber and H. Popper (*Proc. Soc. Exp. Biol. Med.* **74,** *838 (1950)*) and R. C. Goldberg, I. L. Chaikoff, and A. H. Dodge (*Ibid.* **74,** *869 (1950)*). It was shown that the methionine analogue has a destructive effect on the acinar tissue of the pancreas of rats, the extent of this damage being proportional to the dose and duration of the treatment. In extreme cases, the acinar tissue may be completely destroyed and replaced first by infiltrative inflammatory tissue and later by connective tissue.

Ethionine administration also damages hepatic tissue. Early histologic changes, seen two to three days after the beginning of the treatment, consist of fatty degeneration of the liver cells. This is followed a week later by focal necrosis and histiocytic interstitial infiltration. Resistance to the toxicity of ethionine is characterized by sex and species differences, female rats showing more damage than males, and mice being apparently unaffected even by massive doses of ethionine. The destructive effects of ethionine on liver and pancreas can be completely prevented by the simultaneous administration of equal amounts of methionine (Farber and Popper, *loc. cit.*).

That the mode of action of ethionine is through competitive inhibition with methionine is indicated not only by the similarity of some of the symptoms of ethionine toxicity with those of methionine deficiency and by the protective effect of methionine, but also by the demonstration (M. V. Simpson, E. Farber, and H. Tarver, *J. Biol. Chem.* **182,** *81 (1950)*) that ethionine affects protein synthesis by inhibiting the incorporation of methionine and glycine into protein in the liver.

In a recent study (W. Bollag and E. Gallico, *Biochim. et biophys. acta* **9,** *193 (1952)*) previous pathologic observations were confirmed. Furthermore, it was shown that decreases in tissue enzyme concentrations due to ethionine treatment could be demonstrated even before any morphologic change was detectable. Bollag and Gallico used albino stock rats, 8 weeks old, fed a diet containing 15 per cent protein. A number of dosages of ethionine were utilized for various lengths of time. The ethionine was injected intraperitoneally once daily. After the animals were killed, the pancreatic and hepatic tissues were examined histologically, and pancreatic catalase and diastase, and liver catalase activities were measured.

It was found that, as might be expected, pancreatic diastase production ceased following destruction of the acinar tissue by treatment with ethionine. More surprising, production of this enzyme is drastically reduced by moderate doses of ethionine even in areas where acinar tissue appears intact. Generally speaking, the decrease in diastase values corresponds to the dose and duration of treatment.

The catalase activity of the pancreas, normally very low, is somewhat increased by ethionine treatment. This may be a secondary result of inflammatory processes. By contrast, liver catalase is markedly reduced even after a single dose of ethionine.

Bollag and Gallico recall that liver catalase depression is observed in tumor-bearing animals (D. H. Adams, *Brit. J. Cancer* **4,** *183 (1950)*; J. P. Greenstein, "Biochemistry of Cancer," *Academic Press, New York (1947)*). The syndrome showing the most striking analogy with ethionine toxicity, however, seems to be kwashiorkor (*Nutrition Reviews* **9,** *129, 225 (1951)*; **10,** *261 (1952)*), which is characterized by pancreatic and hepatic lesions in many points similar to those produced by administration of ethionine.

Methionine deficiency has been suggested as one of the possible primary etiologic factors in the development of kwashiorkor (J. F. Brock and M. Autret, "Kwashiorkor in Africa," *FAO Nutritional Studies, No. 8, Rome (1952)*). Besides its intrinsic usefulness as a tool in biochemical and histologic studies of methionine deficiency, ethionine may thus prove to be of importance in elucidating the mechanism of development of one of the most widespread and serious nutritional syndromes.

DIETARY PROTEIN AND LIVER XANTHINE OXIDASE ACTIVITY

The liver xanthine oxidase activity in rats can serve as a method for determining the value of dietary protein, according to recent work at the University of Wisconsin (G. Litwack, J. N. Williams, L. Chen, and C. A. Elvehjem, *J. Nutrition* **47,** *299 (1952)*). Earlier results had indicated that this enzyme is very sensitive to the availability of amino acids in proteins (Williams and Elvehjem, *J. Biol. Chem.* **181,** *559 (1949)*) and to deficiencies of amino acids such as methionine and tryptophan (Williams, A. E. Denton, and Elvehjem, *Proc. Soc. Exp. Biol. Med.* **72,** *386 (1949)*; Williams and Elvehjem, *J. Biol. Chem.* **183,** *539 (1950)*).

In the recent report Litwack and associates compared the liver xanthine oxidase activity in adult rats fed meat and other proteins (casein, lactalbumin, gliadin). Over 400 rats were used in the study. The proteins were fed at levels of 5, 10, 18, and 30 per cent of the diet for periods of one, three, five, seven, nine and ten days. The conclusions were based on averages of results obtained on 8 to 18 animals per treatment.

In order to condense the data into usable quantitative form, the enzyme activity on any given day (microliters of oxygen used per hour per gram of fresh liver or per gram of liver nitrogen) was plotted against the level of dietary protein fed. The slopes of the curves (calculated by the method of least squares) indicated that feeding periods of nine to ten days were sufficient to establish an equilibrium between rate of change of enzyme activity and the concentration of dietary protein. Data obtained after a nine-day feeding period thus were used to evaluate the effectiveness of the proteins in maintaining liver xanthine oxidase activity. The enzyme activities (microliters of oxygen per hour per gram of fresh liver) for the proteins studied were as follows: meat protein, 7; casein, 5.5; lactalbumin, 5.0; and gliadin, 0.6. Meat protein was most effective in maintaining liver xanthine oxidase activity; casein and lactalbumin were nearly as good, while gliadin was poor.

Additional studies were conducted to determine if the enzyme data might be correlated with the ability of these proteins to support growth in weanling rats. From the growth data (change in body weight plotted against protein concentration in the diet) lactalbumin appeared to be slightly better than casein, although the two appeared to be almost equal, as was the case when enzyme activity served as a measure of their biologic quality. Gliadin barely maintained initial body weights, regardless of the dietary level used.

The Wisconsin workers point out that the enzyme measurements are probably more specific for evaluating proteins than body weight change since the enzyme studied is itself a protein. It should be pointed out, however, that the protein needs (amino acid pattern) for adult rats may be different from those for growth in young rats (*Nutrition Reviews* **8,** *248 (1950)*). An extension of the Wisconsin study, correlating enzyme activity in young rats with growth rate in young rats, should determine the importance of such differences.

Liver xanthine oxidase is apparently more sensitive to dietary protein than are some other enzymes. G. Litwack, J. N. Williams, P. Feigelson, and C. A. Elvehjem (*J. Biol. Chem.* **187,** *605 (1950)*) noted that loss of activity of certain other liver proteins and of flavin adenine dinucleotide coenzyme proceeded at a slower rate than that of xanthine oxidase.

Liver protein concentration has been used as a measure of protein quality and was thought to serve as a highly sensitive measure of protein quality (R. M. Campbell and H. W. Kosterlitz, *J. Physiol.* **107,** *383 (1948)*). The Wisconsin studies indicate that enzymes, which are themselves proteins, can serve as delicate measures of protein quality and that xanthine oxidase is an enzyme that is particularly adapted for such studies.

INTESTINAL FUNCTION AND ALIMENTATION AFTER RADIATION INJURY

Malnutrition is a frequently observed complication of serious radiation injury. Among the factors important in the pathogenesis of the nutritional deterioration are disturbances in the gastrointestinal tract of animals and human beings exposed to radiation. Pathologic changes (congestion, edema, hemorrhages and ulcers) of the intestinal tract, as well as nausea, vomiting, anorexia, distention and diarrhea are prominent features of the radiation syndrome. The time of onset and severity of the gastrointestinal symptoms depend on the dose of radiation and part of body irradiated.

Decreased gastric acidity and peptic activity, inhibition of respiration of the small intestine, decreased gastric emptying time and increased small bowel activity have been described. R. D. Goodman, A. E. Lewis, and E. A. Schuck (*Am. J. Physiol.* **169,** *242 (1952)*) have recently reported a quantitative study on the net propulsion and absorption availability of gastrointestinal contents in irradiated animals. Their method of study consisted of the administration of a nonabsorbable dye, Evans' blue, into the stomach of rats by means of intubation. At various times thereafter the animals were killed and the dye content of the stomach, small intestines and colon were determined separately. Gastrointestinal transit is defined by Goodman and his colleagues as that fraction of the quantity of material contained in a given segment which is delivered to its adjacent distal segment per unit of time.

Male Wistar strain rats (about 210 g. in weight) were maintained on a standard commercial pellet diet. The irradiated groups were exposed to 450 r total body, single dose, x-irradiation. Observations were made at intervals of one, two, four, seven, ten, fourteen, and twenty-one days after irradiation. A decrease in gastric emptying time was observed within twenty-four hours after irradiation and reached its lowest point (50 per cent below normal) forty-eight hours after exposure. The gastric emptying time remained low for the first ten days, then gradually returned to normal by the fourteenth day. The transit time from the pylorus to the ileocecal junction was not significantly affected. The decrease in absorption availability from the small intestine observed after irradiation was due, then, to a delay in gastric emptying time, rather than to any changes in small bowel motility.

These findings are in keeping with the observations of J. F. Mead, A. B. Decker, and L. R. Bennett (*J. Nutrition* **43,** *485 (1951)*) in regard to the effect of irradiation on fat absorption. They found that in mice which received 600 to 700 r x-irradiation and were fed a meal of fat at either two, twenty-four, or ninety-six hours postirradiation, there was decreased gastric emptying, increased intestinal motility, and little if any

impairment of fat absorption from the small bowel when a correction for the delay in gastric emptying was made.

L. R. Bennett, S. M. Chastain, A. B. Decker, and J. F. Mead (*Proc. Soc. Exp. Biol. Med.* **77,** *715 (1951)*) have reported similar observations in regard to protein absorption. Human serum albumin iodinated with I^{131} was used as the tracer protein. Female mice of the CF_1 strain weighing, on the average, about 28 g., were maintained on a standard stock diet. The test protein was introduced into the esophagus and at varying times thereafter animals were killed by chloroform inhalations. The gastrointestinal tracts were divided into three segments (at the pylorus and at the ileocecal valve) and the contents of each segment were analyzed separately for I^{131} activity. In a number of animals, the radioactivity of the serum protein-bound iodine was also measured. The irradiated test animals were exposed to 600 r x-irradiation in a single dose. This exposure is somewhat greater than the mid-lethal dose. In all irradiated animals there was marked gastric retention during the first twenty-four hours. By ninety-six hours after irradiation, gastric emptying time was normal. When the absorption of protein, as judged by residual I^{131} activity in the small bowel contents, was calculated on the amount of protein reaching the small intestines, it was found to be normal. Nor was there any evidence of impaired permeability of the intestinal mucosa of the irradiated animals leading to large particle absorption as judged by the radioactivity of the serum protein-bound iodine fraction. Bennett *et al.* point out that the absorption rates measured in this study are really those of hydrolysis of protein and absorption of iodotyrosines, and the possibility exists that the absorption of the latter is not representative of all amino acids. However, it would appear, that, as in the case of fat, changes in gastrointestinal motility and tonicity, rather than changes in the function in the intestinal mucosa, are responsible for delayed absorption in the early postirradiation period.

It has been suggested that a reduced consumption of food is responsible in large part for the nutritional depletion seen after irradiation injury. Since the nutritional depletion may lead to an increased morbidity and mortality, efforts directed towards mitigating the malnutrition are indicated.

The effects of forced oral feeding and parenteral alimentation are currently under investigation. W. W. Smith, I. B. Ackermann, and F. Smith (*Am. J. Physiol.* **168,** *382 (1952)*) have recently observed that the forced feeding by stomach tube of a partially predigested diet (principally amigen and dextrose) during the first three days after injury was detrimental to rats exposed to 750 r single dose total body irradiation. The irradiated tube-fed animals showed greatly distended abdomens (chiefly due to gastric stasis) and the percentage of rats surviving was significantly lower than in the control groups which were similarly irradiated, but not tube fed. Tube feedings begun after the fourth day postirradiation were not damaging.

D. E. Smith, E. B. Tyree, H. M. Patt, and N. Bink (*Fed. Proc.* **11,** *149 (1952)*) briefly report that the forced feeding of a semifluid diet (composition not stated) combined with cysteine pretreatment reduced postirradiation weight loss in rats exposed to 800 r total body, single dose, x-irradiation. The forced feeding of either the semifluid diet or a protein hydrolysate-dextrose preparation (amigen), or the subcutaneous injection of the latter, was followed by death of all rats within four days after exposure to the 800 r x-irradiation. Normally, this amount of irradiation results in an 80 per cent mortality.

F. L. Jennings (*Proc. Soc. Exp. Biol. Med.* **80,** *10 (1952)*) has recently studied the comparative efficiency of protein hydrolysate given orally or subcutaneously in respect to weight gain and survival following total body x-irradiation. Sprague-Dawley

male rats weighing about 200 g. were placed on the low protein dietary regimen developed by Cannon and his associates. The rats gradually became protein-deficient, and at the end of ten weeks had lost, on the average, 25 per cent of their body weight. At this time all the animals were exposed to 450 to 650 r single dose, total body x-irradiation. All animals received, orally, the basal low protein diet, and in addition, 1.8 g. daily of protein hydrolysate (amigen), equivalent to 1.35 g. protein per day, orally or subcutaneously. Two thirds of the animals received protein hydrolysate orally; one half of these animals also received 6 ml. saline subcutaneously three times a day. One third of the animals received no oral supplement, but were given 6 ml. of 10 per cent amigen subcutaneously three times a day.

The chief difference among the various groups was the lack of weight loss in the first three to four days following irradiation in the group given amigen subcutaneously as contrasted with the two groups fed orally. Jennings suggests that the difference may possibly reflect an impairment in intestinal absorption during the first three or four days in the groups fed their supplements orally. He does not feel that the early weight increase in the animals receiving amigen subcutaneously is due to water retention since a similar rise was not observed in the group injected with saline. After the fourth day, there was no essential difference in weight recovery whether the protein hydrolysate was given orally or subcutaneously. This suggests that after the fourth day postirradiation, protein utilization is similar in the protein-depleted irradiated rat whether the hydrolysate is given orally or parenterally.

There were no significant consistent differences among the groups in regard to mortality. The $LD_{50/30}$ for the animals given amigen subcutaneously was about 544 r, for the animals given saline subcutaneously 554 r, and for those given no injections about 547 r.

The data of Jennings suggest that although the parenteral feeding of protein hydrolysates during radiation recovery offers no advantage over oral feedings in respect to long-term weight gain and mortality in rats, there is a decrease in weight loss in the early postirradiation period when parenteral feedings are given. Were the parenteral feedings nutritionally complete, the benefits might well be more striking.

Relatively little information is available regarding specific metabolic derangements and nutritional requirements of individuals exposed to radiation. What data are available suggest, in general terms, that individuals in good nutritional state at the time of injury resist radiation injury better than those in poor nutritional states; that morbidity and mortality are less in individuals whose nutritional states are maintained at high levels after injury. However, the optimal quantities and proportions of the various foodstuffs have not been established, nor have the optimal timing and routes of the dietary regimens been determined. Studies in these fields are needed.

TYPE OF FLUID INTAKE AND NUTRITIONAL CHANGES IN RATS

The possible alterations in nutritional status and in the physical sequelae of aging resulting from prolonged use of certain types and quantities of fluids have not as yet been delineated. A recent attempt to compare the effects of different fluids and differing intake volumes is that of C. M. McCay *et al.* (*J. Gerontol.* **7**, *161 (1952)*). These workers have studied groups of rats given different fluids from weaning until death, including investigations of the various regimens upon life span, body weight and skeletal and soft tissue changes.

White rats were divided into four groups

receiving diets as follows: (1) cow's milk supplemented with manganese, copper, iron, iodine and cod liver oil, (2) 10 per cent sucrose as the only fluid, plus a stock diet, (3) coffee as the only fluid plus a stock diet fortified with sucrose to make it calorically equal to the second group's intake, and (4) a similar regimen with water instead of coffee. With these dietary routines the proportions of the various constituents were approximately the same except for the milk-fed rats who received 20 per cent protein instead of the 14 per cent fed to the other groups.

At all ages and at the maximum weights attained, the milk-fed rats had the lowest average weight while the highest weight average was observed in the sucrose-fed animals who also had the greatest body fat. These differences were believed by the authors to be due to higher caloric intake as a consequence of the large amounts of sucrose solution drunk by this group of animals. No difference between the water- and coffee-drinking rats was noted. Correlating significantly with the maximum body weight attained was the life span of the various animals. When analyzed with separation of males and females, longer life was observed in the larger animals and this was found to be related to bone size (humerus), indicating that actual growth rather than adiposity contributed to longevity. As a group, however, females tended to outlive males. Despite the fact that greater body size was attained by the sucrose-fed rats the authors state that they were unable to find any difference in life span between the various groups. This discrepancy remains unexplained and casts some doubt upon the significance of the body size-life span relationship. A similar discrepancy, of course, exists in the fact that the milk-fed animals which had the smallest average body weight were not shorter-lived than the other animals.

Actual volume of fluid consumed was greatest in the milk-fed rats which at the end of five hundred and fifty days had an average daily intake among the males of 24.4 ml. per 100 g. of body weight. Intakes of the other male groups were: sucrose 17.9 ml., coffee 5.3 ml., and water 6.0 ml. Among the females of each group the intakes were slightly higher, being more pronounced in the milk-fed rats. No diuretic effect of coffee which might have been reflected by increased fluid drinking was observed. As the animals aged, there was a decrease in the fluid intake, but this was less marked in the sucrose solution group than in the others.

In terms of total volumes of fluid ingested, the females, as noted above, drank consistently more than the males. Although this may have been compensated for, in regard to caloric intake, by lesser food intakes in the sucrose solution animals, in the animals receiving only milk this reflected a higher caloric intake among females than males. Smaller body weights attained by the females despite their greater calorie consumption suggests a higher basal metabolism than in males. In the milk-fed animals the consumption of calories was found to be approximately three times the resting metabolism determined in rats receiving milk in other studies (S. Brody, "Bioenergetics and Growth," *Reinhold Publishing Co., New York (1945)*), but no resting metabolism studies were performed in this investigation.

All regimens produced positive calcium balance and the calcium intake of the sucrose solution animals was more than twice that of the milk-fed animals. A greater portion of the calcium ingested was excreted in the urine by the latter group than by any of the others, suggesting better absorption of calcium from milk than from the stock diet. Animals receiving coffee or water ingested about twice as much calcium as those given milk, and excreted far less calcium in their urine. No difference was observed in calcium metabolism attributable to coffee as opposed to water drinking. In connection with these calcium metabolism studies the authors noted that the incidence of calcification of

kidneys was greater in females and most strikingly increased by the milk diet in males, with twice as many milk-diet males having renal calcification as any other group of this sex. These findings apparently bore no relation to life span, however, and renal calcification increased with age in all groups. Likewise, calcification of testes and heart was increased with age, but no figures for these organs were reported for the individual groups.

Greatest bone density and calcium content was found in the milk-fed rats of both sexes with higher calcium content being consistently seen in females. Male rats receiving milk, however, had a greater advantage in bone calcium content over the males of other groups than did the females receiving milk. The authors aptly note that since these animals were not bred, the effects of lactation and pregnancy, which could have reduced the sex differential, were not measured by this study. Tooth decay incidence was determined by studies of the lower molars at death and revealed that no decay was found in milk-fed rats, while the incidence varied from 2.4 to 3.8 molars per rat in the other groups. Forty per cent of the sucrose-fed rats had no decayed teeth, while this figure was 18 and 30 per cent for the coffee- and water-fed animals respectively, thus suggesting that susceptible animals in the sucrose group showed markedly higher numbers of decayed teeth per individual than was true of the other groups. This problem of strain variation and caries susceptibility has been previously reviewed (*Nutrition Reviews* **10,** *276 (1952)*) and the findings noted above should be considered of only slight significance until further information is available about the animals used.

This investigation demonstrates in rats the lack of deleterious effect from the continued ingestion of coffee under the circumstances of this study. It also demonstrates the efficacy of supplemented milk as the sole diet for rats and as a means of producing good calcification and density of bones, although body size and weight are best promoted by the use of sucrose and a stock diet. Upon the problems of life span and aging changes and their alteration by diet, however, this investigation yields slight and probably negative results. The implications of its findings and the conclusions to be drawn from it are as yet obscure and should probably not be applied to the effects of continued ingestion of milk, coffee, or sucrose solutions in man.

PLASMA PEPSINOGEN AND ITS RELATION TO GASTRIC SECRETORY ACTIVITY

A proteolytic enzyme, uropepsin, is excreted in the urine of man and experimental animals. The rate of excretion of uropepsin in normal human subjects has been found to be relatively constant. Following surgical removal of the stomach the quantity excreted decreases. There is a similar low rate of excretion in patients with pernicious anemia. In contrast, uropepsin excretion is increased in patients with peptic ulcer. This, and other evidence in the literature, supports the view that uropepsin is related to, and perhaps derived from, secretory mechanisms of the mucosa of the stomach (I. A. Mirsky, S. Block, S. Osher, and R. H. Broh-Kahn, *J. Clin. Invest.* **27,** *818 (1948)*; C. J. Podore, Broh-Kahn, and Mirsky, *Ibid.* **27,** *834 (1948)*). Physicochemical characteristics of the urinary enzyme indicate that it is either identical with or closely related in properties to pepsinogen. Furthermore, it has been found that the quantity of uropepsin in the urine is increased when pepsinogen is administered intravenously to dogs (*Nutrition Reviews* **8,** *309 (1950)*).

If this proteolytic enzyme which appears in the urine is derived from pepsinogen elaborated by the gastric mucosa, it must be assumed that the enzyme is transported in the blood stream and excreted by the kidney. Relatively little is known concerning the circulatory phase of this cycle and the proteases of blood plasma. In an effort to seek this type of information, I. A. Mirsky, P. Futterman, S. Kaplan, and R. H. Broh-Kahn (*J. Lab. Clin. Med.* **40,** *17 (1952)*) have investigated the enzymes responsible for the proteolytic activity of plasma and have described a method for the determination of pepsinogen in plasma.

Proteolytic activity was determined by combining plasma with a substrate of hemoglobin, incubating, and analyzing for the tryosine-like substance released. Reactions were carried out in an acid range of pH, varying from 1.5 to 4.5. Samples of plasma were obtained from healthy subjects, patients following total gastrectomy, and patients with pernicious anemia. By these means it was ascertained that there are at least three enzymes with proteolytic activity in the plasma of normal subjects. The first of these is active at a pH of 1.5 to 3.0. Since most of this activity is lost in the plasma of patients whose stomachs have been removed or who have pernicious anemia, this enzyme system would seem to be dependent on an intact and normally functioning gastric mucosa. A second protease of plasma was found to have maximal activity at pH 3.5 to 4.0. Since this enzyme was present and as active in the plasma of gastrectomized individuals and patients with pernicious anemia as in normal subjects, it apparently is not dependent on a normally functioning gastric mucosa. The third proteolytic system, like the first, has maximal activity at pH 1.5 to 3.0. Evidence of its presence was derived from the relatively small but persistent activity (15 to 30 per cent) in this pH range in plasma of patients whose stomachs had been removed or in the plasma of normal subjects after alkalinization of a previously acidified plasma, a treatment which destroys pepsinogen.

The first of the enzymes possesses the properties of pepsinogen which is autocatalytically converted to pepsin at pH 1.5. The second system is considered to be a protease of the cathepsin type. It is inactivated at pH 1.5 and is apparently derived from tissues other than the stomach. The third enzyme has not been further identified, but does not have its source in the gastric mucosa.

Having thus ascertained the types of proteolytic enzymes present in plasma, the authors were able to develop an apparently effective and reproducible procedure of assay for plasma pepsinogen. The method was applied in the study of plasma pepsinogen in normal subjects and in patients with duodenal ulcer and pernicious anemia (I. A. Mirsky, P. Futterman, and S. Kaplan, *J. Lab. Clin. Med.* **40,** *188 (1952)*). Plasma pepsinogen content was found to be fairly constant in individuals from the group of normal subjects, with relatively little variation in results of determinations done at two- to four-hour intervals throughout the day or at daily intervals for periods of one month. In addition, the ingestion of food did not affect significantly the level of pepsinogen in the plasma, since there was no change in the concentration of the enzyme in samples of plasma obtained at intervals of thirty or sixty minutes during periods of four hours before and after the provision of isocaloric quantities of carbohydrate, protein, or fat, or a mixture of these foods.

Determinations of plasma pepsinogen were done on 312 normal subjects, distributed nearly equally between the two sexes. Although there was a tendency for values to be higher in male subjects than in females, the differences were not statistically significant. In contrast, the activity of this enzyme in plasma is demonstrated to increase with age until the third decade of life. Results of analyses were expressed as micrograms of "tyrosine" released by the

activity of one milliliter of plasma. The mean value for individuals less than 10 years of age was of the order of 400 micrograms per milliliter as compared with 600 micrograms per milliliter in other individuals aged 20 to 29 years, an average increase of about 50 per cent in the two decades. After the third decade plasma pepsinogen content remains relatively constant.

The gastric secretory activity of patients with peptic ulcer is characteristically increased. A group of 98 patients with duodenal ulcer were, therefore, included in the group studied. These patients varied from 20 years to 80 years of age. In all age groups the mean pepsinogen content of plasma was significantly greater than in normal subjects. Moreover, there was a marked tendency for patients with duodenal ulcer to have increasing values in the later decades of life. Not all of the patients had manifestations of active ulcers at the time of the study. Although there was a tendency for higher values among patients with ulcer activity, the differences were not significantly greater in this group than in those with recent history of peptic ulcer, but without currently manifest evidences of activity. It would appear that plasma pepsinogen is increased for long periods and remains so, although the patient may be free of symptoms.

In pernicious anemia the gastric mucosa is characteristically atrophic and the secretory activity of the stomach is depressed. The plasma pepsinogen of 101 patients with this disease was determined and found to be markedly decreased. Mean values in all age groups were approximately 250 micrograms tyrosine per milliliter of plasma. These compared with mean values of from 600 to 700 micrograms per milliliter in normal subjects. The authors suggest that determination of plasma pepsinogen may be of assistance in making the diagnosis of pernicious anemia. All of the patients included in this study had been demonstrated to have the characteristic hematologic changes of this disease, but were under specific therapy when the plasma enzymes were determined. Despite the fact that hematologic changes may have been totally or partially corrected, the decrease in plasma pepsinogen persisted. No data are given on untreated patients.

These observations are of singular interest. The evidence presented seems to demonstrate quite unequivocally that one of the enzymes with proteolytic activity in plasma is derived from the stomach and may be identified as pepsinogen. This would indicate that cells of the gastric mucosa which elaborate this enzyme secrete it not only into the lumen of the stomach but also release it into the blood stream. In contrast to the release of additional quantities of secretions containing pepsinogen into the stomach in response to food, no change has been observed in plasma levels of the enzyme. The significance and functions of this enzyme in the blood are not apparent. The evidence must be accepted that it is excreted in the urine as uropepsin and that levels of pepsinogen in blood and uropepsin in urine provide good evidence of the over-all level of secretory activity of the stomach.

NOTES

Letter to the Editor

Dear Sir:

In the review entitled "Mode of Action of Antibiotics in Poultry Nutrition" that appeared in *Nutrition Reviews* **10,** *270 (1952)* you raise the pertinent point that in the studies of M. E. Coates *et al.* (*J. Sci. Food Agr.* **3,** *43 (1952)*) no attention was given to the possibility of an anthelmintic action of the antibiotics.

May I say on behalf of the authors that, though we did not state this in our paper, our birds were entirely free from ascaridian burden.

M. E. Coates
The National Institute for Research in Dairying
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Berkshire, England

Letter to the Editor

Dear Sir:

The relation of vitamin E to liver necrosis has been reviewed five times in *Nutrition Reviews* (**7,** *302 (1949)*; **8,** *116, 204, 310 (1950)*; **9,** *189 (1951)*). In none of these was reference made to our paper entitled: "A Fatal Vitamin E Deficiency Disease in Rats Characterized by Massive Lung Hemorrhage and Liver Necrosis," E. L. Hove, D. H. Copeland, and W. D. Salmon, *J. Nutrition* **39,** *397 (1949)*. This omission, of course, was a prerogative of your writers.

However, in a more recent review (*Nutrition Reviews* **10,** *149 (1952)*), describing Dr. H. Dam's work with methylene blue, your writer assigned the report of lung hemorrhage as well as liver necrosis to P. György and to K. Schwarz. These workers did not report lung hemorrhage. In fact, they have never used the term in *any* of their publications, to my knowledge. I assume that they have never seen this pathology in their rats.

Our original observation on the occurrence of massive lung hemorrhage in vitamin E-deficient rats on a simple diet with 10 per cent of casein as the protein source has been confirmed by Dr. Dam, and with due reference to our publication. The writer of the above-mentioned review apparently overlooked this point.

Massive lung hemorrhage is a pathologic manifestation of an acute vitamin E deficiency; its occurrence under certain conditions of diet and environment, and not under others, deserves attention. Recently we have noted this condition in dystrophic rabbits, but without evidence of liver damage.

E. L. Hove
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Nutrition Education Filmstrip

A filmstrip, "Let's Teach Better Nutrition," describing the nutrition education research activities of the Department of Nutrition of the Harvard School of Public Health in public schools in Newton, Massachusetts, Rutherford County, Tennessee, and Ascension Parish, Louisiana, has been prepared for school personnel, parent-teacher groups, and community workers to demonstrate how a school community may develop a nutrition education program. The filmstrip is 35 mm., in color, and is available as a filmstrip or slides with or without sound. It may be borrowed for a one-week period at no cost except for transportation by writing to the Department of Nutrition, 695 Huntington Ave., Boston 15, Mass. It is also available for purchase.

CLINICAL NUTRITION BRIEFS

"This type of investigation is of value in regard to the practical feeding of young infants. As the author suggests, the use of more dilute feeding mixtures may be indicated during periods of hot weather, particularly for newborn infants. An obvious alternative would seem to be the offering of water prior to the giving of caloric mixtures to all infants exposed to high temperature environments." (*Nutrition Reviews* **10,** *293* (*1952*), Heat Stress and Food Intake by Infants.)

"The results suggest that certain of the B-vitamins, biotin and niacin in particular, may be furnished under normal conditions to some extent by intestinal bacteria in man." (*Nutrition Reviews* **10,** *294* (*1952*), B-Vitamin Excretion in Man as Influenced by Streptomycin.)

"Reports on the results of liver biopsies performed on newborn babies and infants and children indicate that basic kwashiorkor lesions such as fatty infiltration of the liver and even cirrhosis may, in many cases, date back to some extent from intrauterine life." (*Nutrition Reviews* **10,** *297* (*1952*), Fatty Infiltration of the Liver in Newborn African Babies.)

"Despite the limitations of this study there are included significant evidences of liver dysfunction in markedly obese men." (*Nutrition Reviews* **10,** *325* (*1952*), Association of Liver Damage with Obesity.)

"Infants who must be placed on highly artificial feeding because of allergy to milk or for other reasons deserve very critical analysis of their dietary requirements. Supplementation with those vitamins known to be essential for human nutrition is certainly required and one might reasonably conclude that the addition of a source of the as yet unidentified B-complex factors present in certain crude materials might well be included in such regimens." (*Nutrition Reviews* **10,** *327* (*1952*), Avitaminosis A.)

"The protein intake should be 1.5 to 2 g. per kilogram of body weight per day. Protein protects the liver from damage and promotes its repair. Carbohydrate intake should be as high as possible to supply the bulk of the calories and to exert a protein-sparing effect. Fat should not be restricted below the level needed to allow a palatable diet." (*Nutrition Reviews* **10,** *329* (*1952*), Medical Treatment of Cirrhosis.)

"On the basis of these studies, there are suggestions that an increase in masticatory function may have a beneficial result with respect to the maintenance in normal health of the teeth and their supporting structures." (*Nutrition Reviews* **10,** *333* (*1952*), Masticatory Function of Oral Health.)

"The ingestion of carbohydrate in addition to the distilled water reduced the urine volume by half and also the amount of urine solutes excreted." (*Nutrition Reviews* **10,** *336* (*1952*), Carbohydrate and Seawater in Undernutrition.)